

### **REMARKS**

Favorable reconsideration, reexamination, and allowance of the present patent application are respectfully requested in view of the following remarks and the attached evidence. No new matter is entered.

#### ***Rejection under 35 U.S.C. § 103(a)***

In the Office Action, beginning at page 2, claims 1, 6, 7 and 11-14 were rejected under 35 U.S.C. § 103(a), as reciting subject matters that allegedly are obvious, and therefore allegedly unpatentable, over the disclosure of Kojima in view of the disclosure of Calhoun et al. (hereinafter "Calhoun"), Ciccognani et al. (hereinafter "Ciccognani"), or Kusomoto et al. (hereinafter "Kusomoto") or Sone et al. (hereinafter "Sone"). Applicants respectfully request reconsideration of this rejection.

This rejection and Applicant's arguments against this rejection have been explained and presented in previous Office Actions and responses thereto. Despite applicant's sound arguments presented previously, the Examiner maintains the rejection by combining Kojima, which allegedly discloses L-amino acid fermentation, with Calhoun, Ciccognani, Kusomoto, and Sone, which allegedly suggest NDH-2 disruption, cyo operon-amplification, cytochrome bd oxidase disruption and cytochrome bd oxidase amplification lead to improved growth yield of bacteria. However, as previously argued, improved growth yield does not lead to an improvement in the production of L-amino acids. That is, when the bacterial growth rate is high, new construction of cell components such as cell walls is necessary, and thus more carbon is consumed in the synthesis of cell components, rather than in the biosynthesis of amino acids. One of ordinary skill in the art would understand that carbon flux into L-amino acid synthesis pathways is reduced when cytochrome bo-type oxidase activity is enhanced; and therefore, so one of ordinary skill in the art would never have been motivated to enhance cytochrome bo-type oxidase activity for the production of L-amino acids.

As support for this argument, Applicants submit Exhibit A (Eggeling, et al., Appl. Microbiol. Biotechnol. 1998, 49(1):24-30) and Exhibit B (U.S. Patent No. 5,763,230). Eggeling discloses that the rate of L-lysine excretion is improved by limiting growth

(Tables 2 and 3), and includes the following description in the paragraph bridging from page 29 to page 30.

*"In conclusion, the present study shows that even a classically obtained, very good amino-acid-producing strain can be improved. However, the improvement does not only consist in the removal of a bottleneck, but is due to a subtle flux redistribution at the dehydrogenase/synthase branch point. Combined with this redistribution is an introduced growth limitation, which results in increased availabilities of metabolites within the central metabolism. In fact, for years process engineering has been using extracellular constraints, like limited supply of ammonium or of any medium components to restrict growth, thereby extending the period of increased product accumulation (Kiss and Stephanopoulos 1991; Konstantinov et al. 1991). This is suggested to be due to an increased availability of intracellular precursors. The present case of *dapA* demonstrates a relation of flux increase towards product with an intracellularly introduced growth limitation. Therefore, similar growth limitations, introduced by recombinant DNA techniques, are proposed as an attractive means for the improvement of further metabolite production processes."* (emphasis added).

U.S. Patent No. 5,763,230 discloses that an L-amino acid can be produced efficiently by limiting growth, such as by limiting one or more necessary nutrients (see column 1, line 65 – column 2, line 4):

*"Different types of nutrient limitation can be employed. Carbon source limitation is most often used. Other examples are limitation by the nitrogen source, limitation by oxygen, limitation by a specific nutrient such as a vitamin or an amino acid (in case the microorganism is auxotrophic for such a compound), limitation by sulphur and limitation by phosphorous."*

Column 2, lines 44-51 also can be cited:

*"According to the present process the steady state (or pseudo-steady state in fed-batch culture) is just phosphorous limited, with only a slight accumulation of residual sugar, or just in the region of carbon-phosphorous double limitation with both limiting nutrients practically exhausted. Advantageously the present invention resulted in an improved yield of product on consumed carbon source."*

Finally, column 3, lines 50-55 are also representative:

*“Biomass production is an inevitable by-product of an amino acid fermentation. It is often difficult to find a suitable and economic outlet for this by-product. One of the advantages of the use of phosphorous limitation or phosphorous-carbon double limitation is a great reduction in biomass production.”*

Therefore, one of ordinary skill in the art would never have been motivated to combine Kojima with Calhoun, Ciccognani, Kusomoto and Sone.

Furthermore, even if there was a motivation or even a practical reason to combine Kojima with the other documents, it is quite doubtful from the disclosure of Kojima whether the growth yield could be improved by enhancing cytochrome bo-type oxidase activity in L-amino producing bacteria. That is, Kojima (U.S. Patent No. 5,830,716) discloses in column 3, lines 14-19 *“As for L-amino acid biosynthesis pathways, intravital component which cannot be effectively utilized are often produced through the process of biosynthesis of desired L-amino acids from glucose. It is assumed that such components are ordinarily oxidized through the TCA cycle, resulting in generation of a large amount of NADH.”* Since a large amount of NADH exists in cells of L-amino acid producing bacteria, one of ordinary skill in the art would consider that a sufficient amount of ATP is already present in the cells of L-amino acid producing bacteria, regardless of energy efficiency of respiratory chain pathways and would never expect that growth yield of L-amino acid producing bacteria could be enhanced by modifying the activity of the respiratory chain pathway, much less L-amino acid productivity.

For at least the foregoing reasons, Applicants respectfully submit that the claims, each taken as a whole, would not have been obvious to one of ordinary skill in the art at the time of Applicants' invention, are therefore not unpatentable under 35 U.S.C. § 103(a), and therefore respectfully request withdrawal of the rejection thereof under 35 U.S.C. § 103(a).

***Conclusion***

For at least the foregoing reasons, Applicants respectfully submit that the present patent application is in condition for allowance. An early indication of the allowability of the present patent application is therefore respectfully solicited.

If Examiner Marvich believes that a telephone conference with the undersigned would expedite passage of the present patent application to issue, she is invited to call on the number below.

It is not believed that extensions of time are required, beyond those that may otherwise be provided for in accompanying documents. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and the Commissioner is hereby authorized to charge fees necessitated by this paper, and to credit all refunds and overpayments, to our Deposit Account 50-2821.

Respectfully submitted,

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